

## **Chemistry of Lignocellulose: Methods of Analysis and Consequences of Structure**

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### **ABSTRACT**

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Historically, the chemistry of lignocellulose has been the chemistry of the extraction, solvolysis, hydrolysis and analysis of cellulose and lignin from plant material. The analyses used to determine their relative content are empirical, i.e., the value of the cell wall preparation is defined by the conditions used rather than a molecular entity. As a result, there is great difficulty in comparing the results of analyses of one type of plant to another. This review looks at the ways in which cellulose, lignin and lignocellulose have been chemically isolated and determined and at some of the instrumental methods which can more completely characterize the plant cell wall.

### **INTRODUCTION**

Cellulose is the most abundant compound in the plant world. Lignin gives the cell wall rigidity and renders the lignocellulose complex relatively intractable. Although the history of cellulose and lignin chemistry is old and voluminous, we know little about this vital resource. In 1982, a symposium was held under the auspices of the American Chemical Society on "Wood and Agricultural Residues: Research on Use for Feeds, Fuels and Chemicals" (Soltes, 1983a); virtually all aspects of the structure, reactivity and utilization of cellulose from wood and agricultural residues were discussed. The major emphasis at the symposium was on chemical treatment and evaluation with some emphasis on microbial action. In our workshop, we are stressing the opposite, i.e., we are emphasizing the microbial aspects and letting the chemistry play a supplemental and complementary role in our discussions of the use of agricultural waste materials. This is one of two papers in the workshop that will discuss the chemistry of lignocellulose, in particular methods of analysis and consequences of structure as related to the properties and tractibility of plant cell wall material.

Cellulose and lignocellulose have been described as a homologous polymer with crystalline regions (Thomas, 1983), a semi-crystalline polymer (Attala, 1983), a semi-crystalline polymer with amorphous regions and a highly crystalline polymer (Soltes, 1983b). The form depends on the origin of the cell wall material, how it was obtained and how it was analyzed.

Cellulose is present in all plant cell walls, primary cell walls, middle lamella and secondary cell walls (Shafizadeh and McGinnis, 1971; Cook and Stoddard, 1973). Cellulose accounts for the vast majority of organic matter in plants, followed by hemicellulose and lignin (Barton et al., 1976; Thomas, 1983). Cellulose forms the fibrous skeleton of a plant and is a high molecular weight polysaccharide (Frey-Wyssling, 1954). The lignin "cements" the fibers together and is itself a high molecular weight polymer (Bailey, 1936). Brice and Morrison (1982), in postulating a covalent bond between lignin and hemicellulose, stated that hemicellulose, lignin, and phenolic and acetic acid comprise one vast macromolecular matrix. The binding of cellulose in this vast matrix has been postulated as the primary limiting factor in the utilization or degradation of plant cell walls (Gordon, 1975; Gaillard and Richards, 1975; Hartley and Jones, 1977; Theander et al., 1981) and may be responsible for the observed differential rates of degradation of various forage species (Barton et al., 1976, 1982). The chemistry of lignocellulose is the analytical chemistry of the plant cell wall. To understand the status, a review of the traditional methods of analysis and structural characterization is in order.

#### CHEMICAL METHODS OF STRUCTURAL AND COMPOSITION ANALYSIS

##### *Cellulose*

Cellulose is defined in the "wood" industry as a linear polymer of  $\beta$ -1,4 D-glucose units which form long fibrils and are held together by intramolecular hydrogen bonds (Darvall et al., 1980). Attala (1983) describes the states of aggregation for cellulose as semi-crystalline solids and polymorphic crystalline solids. The distinction being do we describe the aggregate as containing micro-crystalline regions (semi-crystalline) or the individual lattice forms within the polymer (polymorphic). The basic polymorphs are described by their x-ray and/or electron diffraction patterns. The Cellulose I form exhibits a pattern characteristic of the native state of an isolated cellulose, while Cellulose II is that of regenerated cellulose at ambient temperatures. Cellulose III and IV are produced by ammonia treatments, with the regeneration of the cellulose at elevated temperatures, respectively. These definitions precisely characterize cellulose for the wood industry, but are less useful for those of us who work with forages and agricultural residues.

Cellulose preparation from forages has been modified over the years from Crampton and Maynard (1938) true cellulose, through the van Soest (1973)

detergent analysis system. True cellulose is an acetic/nitric acid extraction which leaves a residue that is reported to be 90% glucose (Sullivan, 1969). Natural cellulose, obtained by a procedure which removes lignin via chlorination, contains the cellulose plus some polysaccharides which are part of the hemicelluloses and pectins (Sullivan, 1955). The use of chlorination for delignification is part of the holocellulose preparation which produces a snow-white fibrous fraction (Phillips et al., 1960). Crude fiber, a part of the Weende proximate analysis scheme has also been used to prepare fibrous fractions of which cellulose is the principle component (Association of Official Analytical Chemists, 1980).  $\alpha$ -Cellulose is a residue produced by extraction with 17.5% sodium hydroxide and resembles true cellulose (Sullivan, 1969). Normal acid fiber is obtained after boiling forages with 1.0 N sulfuric acid (Walker and Hepburn, 1955). This particular preparation (i.e., normal acid fiber) may have been a starting point for the detergent analysis system.

The detergent analysis system was developed by van Soest in the 1960s (van Soest, 1967) and consists of an acid detergent fiber fraction, resulting from a 2% hexadecyltrimethyl ammonium bromide in 1.0 N sulfuric acid hot extraction (van Soest, 1963). This fraction can be used to determine lignin by the 72% sulfuric acid method (van Soest, 1963), in which case the carbohydrate is dissolved, or a permanganate oxidation which leaves a cellulose preparation (van Soest and Wine, 1968). A total fiber fraction, referred to as neutral detergent fiber, results from the hot extraction of a forage with a buffered 2% sodium lauryl sulfate solution (van Soest and Wine, 1967). These analyses have become very widely accepted in the last 15–20 years.

### *Lignin*

Lignin, while not as prevalent in nature as cellulose, still accounts for some 12 billion tons of material formed annually; roughly equivalent to the amount of hemicellulose and one quarter of the annual production of cellulose (Lieth, 1973). Lignin is a polyphenol formed by enzyme-initiated dehydrogenative polymerization of phenylpropanoid precursors (Sarkanen and Ludwig, 1971). The products of the shikimic acid pathway, phenylalanine and tyrosine, ultimately yield *p*-coumaryl, coniferyl and sinapyl alcohols which account for most of the lignin molecule (Harkin, 1973). Since the polymerization mechanism is enzyme initiated and free radical propagated, the resulting molecule is complex and seemingly random (Brown, 1964; Harkin, 1973). Softwood lignins are principally coniferyl groups (80%), hardwoods are mostly coniferyl (56%), but contain 40% sinapyl vs. 6% in softwoods (Harkin, 1973). The lignins of grasses are much higher (35–50%) in *p*-coumaryl groups than trees (4–14%) (Himmelsbach and Barton, 1980). The extraction of forages with alkali yields *p*-coumaric, diferulic and *p*-hydroxybenzoic acids and vanillin (Gordon, 1975; Hartley and Jones, 1977).

The highly polymerized and condensed phenylpropanoid matrix has been referred to as "core" lignin (Gordon, 1975). This matrix appears to be linked to the structural carbohydrates that comprise the cell wall by ester linkages (Hartley and Jones, 1977). The other "non-core" lignin may act as cross-links between structural carbohydrates and lignin (Gordon, 1975). This would give strength and rigidity to the plant cell wall.

The work of Morrison (1974) and Gaillard and Richards (1975) has shown alkali-labile bonding between lignin and the structural carbohydrates. An ester linkage would appear to be the most likely type of bond (Morrison, 1973). Apart from the isolation of lignin-carbohydrate complexes, lignin has been assayed in many ways. The original citation for the extraction of lignin from wood pulp with organic solvents, that basis of wood pulping, is the work of Klason (1893). Sarkanen (1980) reviewed the processes by which lignin is separated from the cell wall. Forages and agricultural residues differ from trees in that the amount of lignin and the maturity of the plant at harvest are considerably less. A characterization of lignin in forages by nuclear magnetic resonance (NMR) spectroscopy was made by Himmelsbach and Barton (1980). This study pointed out the differences between cool- and warm-season grasses. In addition, it appears that the larger amount of *p*-coumaryl lignin moieties in warm-season grasses provides more opportunities for cross-linking of structural carbohydrates (Barton et al., 1982). This could provide a rationale for the lower digestibility of warm-season grasses by ruminants (Minson and McLeod, 1970; Barton et al., 1976).

The two methods most in use today to measure lignin are 72% sulfuric acid and permanganate oxidation of acid detergent fiber (ADF) (van Soest, 1963; van Soest and Wine, 1968). Work by Akin et al. (1975), Barton and Akin (1977) and Barton et al. (1981) helped to clarify the fibrous fraction in the detergent analysis procedure and the effects of lignin on the digestion of particular cell wall tissues by rumen microorganisms. Two other ways to remove lignin as a barrier to utilization of cell wall structural carbohydrates are by oxidation with hydrogen peroxide or ozone. Alkaline peroxide has been used to delignify agricultural residues in order to enhance enzymatic saccharification for further processing (Gould, 1984). The use of peroxide-treated lignocellulose residues raises the efficiency of ethanol production to > 90% (Gould and Freer, 1984). Mechanistic studies indicate that hydroxyl free radicals and oxygen radical anions are the oxidizing species in the delignification (Gould, 1985a). Hydrogen peroxide has been used on perennial forage grasses and increases their susceptibility to enzymatic degradation (Gould, 1985b). Conceptually, ozone is the ideal reagent for delignification. It is a gas, highly reactive and easily produced from air and electricity. Ozone has been used to treat cotton and wheat straw and improve its digestibility for ruminants (Ben-Ghedalia et al., 1980, 1982; Ben-Ghedalia and Miron, 1981). The basic problem is that the by-products can be toxic to rumen micro-organisms (Ben-Ghedalia et

al., 1980). The products of lignin degradation, if ozone is allowed to completely degrade the aromatic ring structure, are organic acids, principally oxalic acid derivatives (Bailey, 1936; Ben-Ghedalia and Miron, 1981; Tishchenko, 1959). The work of Bolker and Teraschima (1966) describes the ways in which lignin can be isolated by solvolysis. The preparation of grass lignin as acetals was the solvolysis method used by Himmelsbach and Barton (1980) to describe the structure of isolated lignin fractions.

#### PHYSICAL METHODS OF STRUCTURAL AND COMPOSITION ANALYSIS

Physical methods, both chromatography and spectroscopy, have made tremendous advances. These tools have aided in the study of lignocellulose and plant cell wall carbohydrates. The alditol acetate gas-liquid chromatography (GLC) procedure has been used quite effectively by Darvill et al. (1980) in the study of plant cell wall polysaccharides by the Albersheim group. Their work on isolated polysaccharides has provided evidence for considering small oligomers called oligosaccharins as plant growth regulators (Gollin et al., 1984). The use of high pressure liquid chromatography (HPLC) for carbohydrate analysis was described by Conrad and Palmer (1976). The HPLC method is faster and does not require derivatization. In the analyses of forage hemicellulose, a method was developed to assay the neutral sugars hydrolyzed from neutral detergent fiber (NDF) (Barton et al., 1982; Windham et al., 1983). The utilization of carbohydrates through the ruminant animal has been studied using the HPLC technique to assay the individual sugars (Windham et al., 1986).

The use of NMR spectroscopy and near-infrared reflectance spectroscopy (NIRS) has added new dimensions to analyses of forages for quality (Himmelsbach et al., 1983; Martens et al., 1983). In essence, these two techniques are very complementary. The NMR is readily interpretable and good quantitative information can be obtained. It is, however, slow and expensive. The lignins of forage grasses were examined by NMR and found to contain more unsubstituted lignin moieties than "woody" plants (Himmelsbach and Barton, 1980). The advent of solid-state NMR meant that forages and fibrous residues could be analyzed as solids (Stejskal and Schaefer, 1975; Schaefer and Stejskal, 1979). Himmelsbach et al., (1983) compared solid-state NMR and NIRS and found that the spectroscopy methods agreed very closely and better than either method compared with laboratory wet chemical analysis.

A major research need is the requirement to correlate the results of numerous spectroscopic and chromatographic techniques to give a coherent interpretation of the spectral and chromatographic data as it relates to quality and nutritional determinations. The work of Martens (1983) showed how partial least squares techniques could be used to correlate data from more than one source. A study by Himmelsbach et al. (1986) describes the crystallinity of

cellulose by solid-state NMR, NIRS, scanning electron microscopy (SEM) and Fourier transformed infrared (FT-IR) spectroscopy. The unique abilities of each technique to more fully explain the concept of crystallinity and the relationship to particle size is discussed.

The future of lignocellulose chemistry will be in the area of multiple analytical techniques and chemometric methods. Chemometrics has been defined as the development and application of mathematical and statistical methods to extract useful chemical information from chemical measurements (Kowalski, 1977). These techniques, coupled with the traditional techniques of the microbiologist and microscopist, will help to forge an analytical system to answer agricultural problems. We can no longer afford the luxuries of long laboratory analyses that are both resource and labor intensive. The new rapid analytical capabilities of the biological and chemical scientists will aid in the evaluation of the genetic manipulation of plant and bacterial species to produce better crops and to improve the efficiency of their use.

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